

GenCore version 5.1.6
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OM protein - protein search, using sw model
Run on: September 24, 2004, 13:44:25 ; Search time 125 seconds
(without alignments)
(without alignments)
(without alignments)
560.574 Million cell updates/sec

Title: US-09-554-465-75

Perfect score: 1324

Sequence: BIOSUM62

1. EVOLLEQSGRELVPRGTSVK.....CQNDPSYPLTFGAGTKLKK 248

Scoring table: Gapop 10.0 , Gapext 0.5

Searched: 1586107 seqs, 282547505 residues

Total number of hits satisfying chosen parameters: 1586107

Minimum DB seq length: 0

Maximum DB seq length: 200000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : A_Geneseq_29Jan04:*

- 1: geneseq1980s:*
- 2: geneseq1990s:*
- 3: geneseq2000s:*
- 4: geneseq2001s:*
- 5: geneseq2002s:*
- 6: geneseq2003as:*
- 7: geneseq2003bs:*
- 8: geneseq2004s:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	Query	%	Match length	DB ID	Description
1	1324	100.0	-248	2	AYV17964	Ayv17964 Mouse scf
2	1261	95.2	-248	2	AYV17960	Ayv17960 Mouse scf
3	1203	90.9	-248	2	AYV17965	Ayv17965 Mouse scf
4	1177	80.9	-258	5	AUW72865	Auw72865 P5-2 sing
5	1060	80.1	-242	2	AYV17959	Ayv17959 Mouse scf
6	1057	79.8	-242	2	AYV17957	Ayv17957 Mouse scf
7	1054	79.6	-242	2	AYV17961	Ayv17961 Mouse scf
8	1027	77.6	-258	5	AUW72871	Auw72871 3BLIXP4-3
9	1027	77.6	-505	5	AUW72875	Auw72875 Human NKG
10	1022	77.2	-289	2	RAWB2743	Rawb2743 Fusion pr
11	1022	77.2	-673	2	RAWB2742	Rawb2742 Plasmid p
12	1009	76.2	-242	4	ABR31422	Aab31422 Protein u
13	1004.5	75.9	-262	4	ABR31421	Aab31421 Protein u
14	995.5	75.2	-500	7	ADD13792	Add13792 Plasmid p
15	990.5	74.8	-255	5	ABR72870	Auw72870 P5-23 sin
16	988.5	74.7	-271	4	ABR31423	Aab31423 Protein u
17	988	74.6	-272	4	ABR31424	Aab31424 Protein u
18	978.5	73.9	-259	5	AUW72862	Auw72862 P4-3 sing
19	978	73.9	-44	6	ABU39018	Abu39018 CC49 sing
20	972	73.4	-532	2	AWM5565	Auw5565 HindIII-E
21	956	72.2	-580	2	AWW90217	Auw90217 Bispecific
22	953	72.0	-556	2	AWW90218	Auw90218 Bispecific
23	940.5	71.0	-251	2	AYV17958	Ayv17958 Mouse scf
24	935.5	70.7	-251	2	AYV17962	Ayv17962 Mouse scf
507	935.5	70.7	-507	2	AUW72858	Auw72858 8G7Cl0x4-

ALIGNMENTS

26	935.5	70.7	510	5	AUW2859	Auw72859 6E5A7x4-7
28	935.5	70.7	532	3	AAY78328	Aay78328 Bispecific
29	921	69.6	249	2	RAW60770	Aaw60770 Single ch
30	914	69.0	543	7	ADD13876	Add13876 CD28/mela
31	908.5	68.6	291	4	AAB20443	Aab20443 Antibody
32	908.5	68.6	322	4	AAB20440	Aab20440 Antibody
33	908.5	68.6	729	4	AAB20439	Aab20439 Antibody
34	905	68.4	282	2	AAW0818	Aaw0818 VH475-11
35	905	68.4	282	2	AAM5564	Aam5564 HindIII-E
36	905	68.4	355	2	AAW5133	Aaw5133 R. Pipien
37	902	68.1	483	2	AABW8099	Aaw8099 A protein
38	902	68.1	483	3	AAB07935	Aab07935 A divalent
39	902	68.1	483	3	AAY57254	Aay5725 Divalent
40	902	68.1	483	3	AAB21679	Aab21679 Bivalent
41	902	68.1	483	3	AAYB0924	Aayb0924 Bivalent
42	902	68.1	483	6	AUB61803	Abu61809 Divalent
43	902	68.1	486	2	AAR31649	Aar31649 Sequence
44	898	67.9	392	3	AAB10863	Aab10863 S11-scVEG2
45	898.5	67.9	510	3	AAB10864	Aab10864 S11-scVEG2

Applicant:

The invention relates to a method of identifying binding site domains (BSD) that retain the capacity of binding to a predetermined epitope when positioned C-terminal of at least one further domain in a recombinant bi-polypeptide. The method comprises (a) testing a panel of polypeptides or multivalent polypeptides, the method comprising (a), testing a panel of a fusion protein for binding to a predetermined epitope, where the fusion protein comprises an additional domain positioned N-terminal of the BSD and an amino acid sequence that mediates anchorage of the fusion protein to the surface of the display system; and (b) identifying a BSD that binds to the predetermined epitope. The method is useful to identify bi- or multivalent polypeptides that comprise antibody binding sites capable of efficiently binding to the corresponding antigen. The polypeptides or antibodies identified by the method are useful therapeutically and

CC diagnostically, for e.g. cancer and autoimmune diseases. ScFv-antibody
 CC fragments that bind independently of their position within bifunctional
 CC single-chain fusion proteins can be isolated from combinatorial antibody
 CC libraries using the new in vitro method. Sequences AAY17957-965 represent
 CC mouse scFV fragments
 XX Sequence 248 AA;

Query Match 100.0%; Score 1324; DB 2; Length 248;
 Best Local Similarity 100.0%; Pred. No. 2.7e-87; Mismatches 0; Indels 0; Gaps 0;
 Matches 248; Protein: 248 AA.

Qy 1 EVOLLESGAELVRPGTSVKISKCKASGYAFNYWLGWKGKRPGRHGLEWIDDPGSGNTH 60
 Db 1 EVOLLESGAELVRPGTSVKISKCKASGYAFNYWLGWKGKRPGRHGLEWIDDPGSGNTH 60
 Qy 61 YNEKFKGKATLTADKSSTAYMQLSSLTIFEDSAVFCARLNWDPEMDWQGQTIVTVSS 120
 Db 61 INEKFKGKATLTADKSSTAYMQLSSLTIFEDSAVFCARLNWDPEMDWQGQTIVTVSS 120
 Qy 121 GGGGSGGGGGGGSELVMTOSSPSSITVTGEGKVTCMCSCEQSOSLNSGNKNVLYTQQK 180
 Db 121 GGGGSGGGGGGGSELVMTOSSPSSITVTGEGKVTCMCSCEQSOSLNSGNKNVLYTQQK 180
 Qy 181 PQQPKLILYMASTRSGVPDRFTGSGGDPFTLISVOEDLAVYCONDYSPITFG 240
 Db 181 PQQPKLILYMASTRSGVPDRFTGSGGDPFTLISVOEDLAVYCONDYSPITFG 240
 Qy 241 AGTKLEK 248
 Db 241 AGTKLEK 248

RESULT 2
 AAY17960

ID AAY17960 standard; protein; 248 AA.

XX AC AAY17960;

XX DT 04-AUG-1999 (First entry)

DE Mouse scFv fragment 4-1.

XX KW Binding site domain; BSD; epitope; fusion protein; therapeutic; cancer;

XX OS Mus sp.

XX PN WO9925818-A1.

PD 27-MAY-1999.

XX PR 16-NOV-1998; 98WO-EP007313.

XX PA (KUFE/1) KUFER P.

XX PI Kufner P., Raum T., Borschert K., Zettl F., Lutterbuese R;

XX DR N-PSDB; AAX77243.

PT Phage display system for identification of binding site domains retaining

capacity to bind an epitope.

XX PS Claim 27; Fig 6.6.; 152PP; English.

XX The invention relates to a method of identifying binding site domains
 CC (BSD) that retain the capacity of binding to a predetermined epitope when
 CC positioned C-terminal of at least one further domain in a recombinant bi-
 CC or multivalent polypeptide. The method comprises (a) testing a panel of
 CC BSD displayed on the surface of a biological display system as part of a

fusion protein for binding to a predetermined epitope, where the fusion protein comprises an additional domain positioned N-terminal of the BSD and an amino acid sequence that mediates anchoring of the fusion protein to the surface of the display system; and (b) identifying a BSD that binds to the predetermined epitope. The method is useful to identify bis- or multivalent polypeptides that comprise antibody binding sites capable of efficiently binding to the corresponding antigen. The polypeptides or antibodies identified by the method are useful therapeutically and diagnostically, for e.g. cancer and autoimmune diseases. ScFv-antibody fragments that bind independently of their position within bifunctional single-chain fusion proteins can be isolated from combinatorial antibody libraries using the new in vitro method. Sequences AAY17957-965 represent mouse scFV fragments

Sequence 248 AA;

Query Match 95.2%; Score 1261; DB 2; Length 248;
 Best Local Similarity 95.6%; Pred. No. 9e-83; Mismatches 8; Indels 0; Gaps 0;
 Matches 237; Conservative 3; Protein: 248 AA.

Qy 1 EVOLLESGAELVRPGTSVKISKCKASGYAFNYWLGWKGKRPGRHGLEWIDDPGSGNTH 60
 Db 1 EVOLLESGAELVRPGTSVKISKCKASGYAFNYWLGWKGKRPGRHGLEWIDDPGSGNTH 60
 Qy 61 YNEKFKGKATLTADKSSTAYMQLSSLTIFEDSAVFCARLNWDPEMDWQGQTIVTVSS 120
 Db 61 INEKFKGKATLTADKSSTAYMQLSSLTIFEDSAVFCARLNWDPEMDWQGQTIVTVSS 120
 Qy 121 GGGGSGGGGGGGSELVMTOSSPSSITVTGEGKVTCMCSCEQSOSLNSGNKNVLYTQQK 180
 Db 121 GGGGSGGGGGGGSELVMTOSSPSSITVTGEGKVTCMCSCEQSOSLNSGNKNVLYTQQK 180
 Qy 181 PQQPKLILYMASTRSGVPDRFTGSGGDPFTLISVOEDLAVYCONDYSPITFG 240
 Db 181 PQQPKLILYMASTRSGVPDRFTGSGGDPFTLISVOEDLAVYCONDYSPITFG 240
 Qy 241 AGTKLEK 248
 Db 241 AGTKLEK 248

RESULT 3
 AAY17965

ID AAY17965 standard; protein; 248 AA.

XX AC AAY17965;

XX DT 04-AUG-1999 (first entry)

DB Mouse scFv fragment 5-13.

XX KW Binding site domain; BSD; epitope; fusion protein; therapeutic; cancer;

XX OS Mus sp.

XX PN WO9925818-A1.

PD 27-MAY-1999.

XX PR 16-NOV-1998; 98WO-EP007313.

XX PA (KUFE/1) KUFER P.

XX PI Kufner P., Raum T., Borschert K., Zettl F., Lutterbuese R;

XX DR N-PSDB; AAX77248.

XX PT Phage display system for identification of binding site domains retaining
 capacity to bind an epitope.